Structure of Pex5p and Pex5-20 complexes in the yeast Hansenula polymorpha. Pex20p causes a conformational change upon binding to Pex5p tetramers involved in peroxisomal protein transport.

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Structure of Pex5p and Pex5-20 complexes in the yeast *Hansenula polymorpha*. Pex20p causes a conformational change upon binding to Pex5p tetramers involved in peroxisomal protein transport.

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Peroxisomal matrix proteins are synthesized on free polyribosomes and directed to the organelle by specific peroxisomal targeting signals (PTSs).

The *PEX5* gene encodes the PTS1 receptor, Pex5p, which interacts with the PTS1 signal via a series of tetratricopeptide repeats (TPRs) within its C terminus. A crystal structure has been determined of a 41 kDa fragment of human Pex5p that includes six TPR motifs in complex with a small peptide containing a PTS1 sequence [1,2] or the sterol carrier protein [3]. This structure reveals the molecular basis for PTS1 recognition which is mostly formed by two clusters of three TPRs almost completely surrounding the PTS1-peptide.

However, whether or not Pex5p functions as an oligomer, is still a matter of debate. Gel filtration chromatography and electron microscopy studies indicated that human Pex5p (HsPex5p) is a homotetramer [4]. Fluorescence spectroscopy studies on Pex5p of the yeast *Hansenula polymorpha* (HpPex5p) indicated that HpPex5p also forms oligomers [5].

In this study, the projection structures of HpPex5p and HpPex5p-HpPex20p complexes were investigated by single particle electron microscopy. The analysis shows that HpPex5p is a tetramer and that HpPex20p is able to induce a major conformational change leading to a rather open space in the centre of the HpPex5p tetramer. In a successive set of experiments we show that HpPex5p-HpPex20p complexes are able to bind folded copies of tetrameric catalase at the periphery. Since catalase is one of the major peroxisomal proteins this indicates that such HpPex5p-HpPex20p-catalase complexes are functional as receptor complex.

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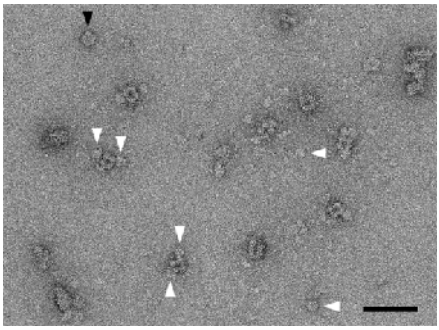

Figure 1. Electron micrograph of negatively stained Pex5p-20p complexes. The black arrowhead points to a Pex5p tetramer in the closed conformation; the white arrowheads indicate Pex20, either attached to Pex5p in the open conformation or as single complexes. The space bar indicates 50 nm.

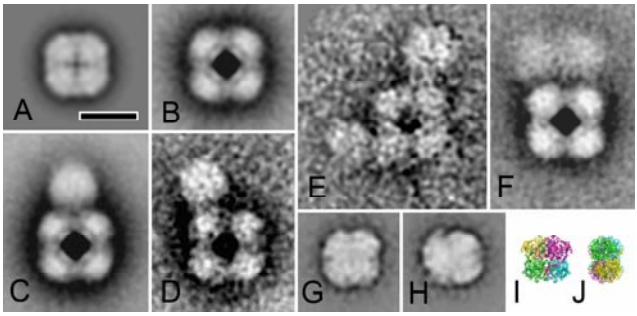

Figure 2. Single particle image analysis of Pex5p, Pex5-20 and Pex5-20-catalase complexes. (A) Average projection map of purified Pex5p in the closed conformation. (B) Average projection map of purified Pex5p in the open conformation. (C) main view of the Pex5-20 complex. (D) averge map of a small class of Pex5-20 complexes in which the upper Pex20 multimer is displaced. (E). Another class of Pex5-20 complexes in which a Pex20 multimer second multimer is binding to the left side of the Pex5p tetramer. (F) main class of Pex5-20-catalase complexes (G,H) main views of purified catalase tetramers. (I) High-resolution catalase X-ray model in a position similar to the EM projection of image G, in which it is slightly tilted out of its 4-fould symmetrical view. (H) side-view of the X-ray model in which two monomers are almost in overlap with two others. Four-fold symmetry was imposed on images of (A) and (B) after completion of analysis. The space bar equals 10 nm.